REVIEW OF LECONTVIRUS

A NUCLEAR POLYHEDROSIS VIRUS

WITH SPECIAL EMPHASIS

ON THE AQUATIC ENVIRONMENT

DRAFT

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Review of Lecontrivus A Nuclear Polyhedrosis Virus With Special Emphasis On The Aquatic Environment

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PREAMBLE

In response to the Ministry of Natural Resources preparation of Class Environmental Assessment for timber management on crown lands, the Ontario Ministry of the Environment commissioned the Environmental Review Of Lecontvirus A Nuclear Polyhedrosis Virus With Special Emphasis On The Aquatic Environment.

This review focuses on the aquatic ecosystem effects of this insecticide as they relate to the use of Lecontvirus in the control of the redheaded pine sawfly (Neodiprion lecontei) on crown lands. The review also considers the effects of Lecontvirus to non-target terrestrial biota (wildlife and humans) as they relate to the exposure of these lifeforms through the contact and ingestion of contaminated water and/or biota.

The intent was to develop a Provincial Water Quality Objective (PWQO) from the information provided in the review document. However, the lack of an analytical method for enumerating Lecontvirus in water has prohibited the development of a numerical PWQO. Instead, a narrative **ENVIRONMENTAL IMPACT STATEMENT** has been prepared and is provided on the following pages.

LECONTVIRUS

STATEMENT OF ENVIRONMENTAL IMPACT

Available toxicological evidence for Lecontvirus and other Baculoviridae to aquatic life, indicates that Lecontvirus would not pose a threat to aquatic life or other beneficial uses of the Province's surface waters from its properly planned, controlled and supervised treatment for the redheaded pine sawfly (Neodiprion lecontei).

RATIONALE

INTRODUCTION

Lecontvirus is a biological insecticide registered in Canada, under the Federal Pest Control Products Act in 1987 (PCP# 17824) for the control of the red-headed pine sawfly Neodiprion lecontei (Fitch). The insecticide actually consists of diseased N. lecontei larvae infected with a nuclear polyhedrosis virus (NPV). This virus belongs to the family Baculoviridae subgroup A (embedded DNA virus in large numbers of polyhedral inclusion bodies of protein).

Lecontvirus is only effective against the red headed pine sawfly, N. lecontei. No organisms outside the family Diprionidae, of which the red-headed pine sawfly is a member, are known to be affected by Lecontvirus. Lecontvirus must be ingested and enter the larval gut where the alkaline pH conditions dissolves the protein inclusion bodies and thereby releases the infectious virions. These virions penetrate the cells of the gut and initiate cellular infection which leads to the death of the target organism in 10 to 12 days. The larvae generally cease feeding well in advance of mortality.

The use of Lecontvirus in Canada is restricted for application under the direct supervision of the federal or provincial forestry service. It is used to protect plantations of red pine, jack pine and Scots pine, and is generally only applied to trees less than 15 meters high.

ENVIRONMENTAL FATE

Little research on the environmental fate of Lecontvirus exists since there is no method to quantify residues. Since the virus exists naturally, it must be capable of overwintering. Baculoviruses can persist for several years in soil and it is reasonable to assume that Lecontvirus can also persist for the same length of time. Corpses of infected larvae adhering to trees and NPV contaminated bird faeces are possible sources and may aid in

disseminating the virus in the environment. The most probable route by which Lecontvirus may enter the aquatic environment is through spray drift and surface run-off during and after application. However, plantation sites are typically located on sandy soils generally removed from bodies of water. In addition, the small quantity of polyhedral inclusion bodies applied per hectare would result in minimal exposure of the aquatic environment.

EFFECTS ON AQUATIC LIFE

Due to the specificity of Lecontvirus, few studies on the toxicity to aquatic organisms exist. Hicks et al. (1981) found no effects in toxicity tests exposing rainbow trout and Daphnia pulex for 21 and 14 days respectively to dosages of Lecontvirus well in excess of amounts of material which could enter streams and water bodies. In addition, Kingsbury et al. (1978) failed to detect any impact on aquatic invertebrates in operational field trials. Tests conducted with a range of aquatic invertebrates and fish to other Baculoviridae (e.g. Heliothis NPV) indicated no effects.

EFFECTS ON TERRESTRIAL LIFE

A range of terrestrial animals have been exposed to Lecontvirus via topical applications, intubation and through inhalation routes to levels well in excess of those that could occur from applications to plantations with no effects observed. The only confirmed effect noted for Baculoviruses on vertebrates, has been slight skin or eye irritation in rabbits with viruses other than Lecontvirus. The effect was believed to be due to the insect material (ground insect parts) associated with the virus, and not the virus itself.

HUMAN HEALTH EFFECTS

From the available animal data specific to Lecontvirus, human data related to other baculoviruses, and the margin of safety tested, there appears to be no concern for humans from exposure to Lecontvirus.

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CONCLUSIONS

1) The scientific literature indicates that all baculoviruses, including Lecontvirus, are specific to the mid-gut epithelial cells of their host insect. The key to safety with the use of Lecontvirus is its inherent host-specificity.

2) In Ontario, use of Lecontvirus is minor. Approximately 2400 hectares of pine plantations have been treated by ground application between 1980-1987.

3) The impact of Lecontvirus to organisms other than Neodiprion lecontei appears to be undetectable, based on all available scientific data.

4) The use of Lecontvirus does not represent a detectable impact to the quality of surface waters in the Province of Ontario.

1.0 INTRODUCTION

The Ontario Ministry of Natural Resources has recently completed a draft, Class Environmental Assessment for timber management on crown lands. Under this Class Environmental Assessment, the Ministry of Natural Resources proposes (when required) to use the nuclear polyhedrosis virus, Lecontvirus, for control of the red-headed pine sawfly, Neodiprion lecontei.

The potential for this "biological" insecticide to enter surface water during and after application to timber lots is of potential concern to the Ontario Ministry of the Environment. The Ontario Ministry of the Environment has been mandated to develop and, where appropriate, revise the Provincial Water Quality Objectives and Policies to protect the province's water resources. These water quality objectives and policies are designed to assure that "surface waters in the province are of a quality which is satisfactory for aquatic life and recreation."

In order to formulate a sound environmental policy for Lecontvirus, the available scientific data must be procured and analyzed. The purpose of this document is to provide the current information known about this compound with particular emphasis on protecting surface waters.

2.0 LECONTVIRUS

2.1 General Description

Lecontvirus is a biological insecticide, given temporary registration in 1983 and full registration in 1987 (PCP #17824; Appendix 1), for control of the red-headed pine sawfly Neodiprion lecontei (Fitch) in Canada. The insecticide actually consists of diseased N. lecontei larvae infected with a nuclear polyhedrosis virus. This virus belongs to the family Baculoviridae subgroup A (embedded DNA virus in large numbers of polyhedral bodies of protein). Larvae infected with virus are frozen, lyophilized and ground to a fine powder. Actual virus represents about 0.05% of the preparation, the rest being milled insect parts (Cunningham et al. 1986). The material is generally supplied in a suspension in emulsifiable oil which is then diluted in water. The oil formulation is made with Dipel 80 and the formulated product contains 5×10^8 polyhedral bodies/ml.

The particles which cause infection (virions) are rod-shaped, contain DNA, and are embedded within rod-shaped proteinaceous crystal bodies known as polyhedral inclusion bodies (PIB's). The PIB's have a mean diameter of 0.72 um (range 0.29-1.44 um). The insecticide contains a minimum of 5×10^8 PIB's/ml of product. It is the living, replicating virions, and not toxic agents, which cause mortality of larvae.

2.2 Production Process

The registrant for Lecontvirus is the Forest Pest Management Institute, Box 490, Sault Ste. Marie, Ontario P6A 5M7. The actual contact

for production of the virus is Dr. John Cunningham at the Institute (705-949-9461).

Unlike most other insecticides, the production of this virus is done in a research facility by Dr. Cunningham and technical staff. The production methods for the virus have been outlined by Cunningham and McPhee (1986; Appendix 2). Basically, healthy N. lecontei larvae (laboratory-reared or field-collected) are infected with NPV virus. Larvae are fed on pine foliage, since no artificial diets exist. The larvae are harvested 10 to 12 days later (allowing time for viral replication) (Anonymous 1987). The diseased larvae are frozen, gently dried, and ground to a fine powder.

This powder contains 0.05% virus particles and 99.95% finely-milled insect parts. The ground larvae form a grey powder whereas purified polyhedras are white. The material is soluble in both oil and water with a pH between 6 to 8. Ground larvae may be stored for several years without loss of activity at 4°C, but formulated material (i.e. in oil) should be stored no more than 2 months. Each season, Dr. Cunningham formulates new material upon request from forest service personnel.

2.3 Impurities in Commercial Products

Commercial Lecontvirus contains virus particles and ground insect parts. These ground insect parts may be considered impurities and obviously contain a wide spectrum of proteins, carbohydrates, lipids, chitin and potential bacterial contaminants. In sterility checks of Lecontvirus preparations, Forsberg et al. (1978) found Enterobacter agglomerans in healthy larvae and E. agglomerans, E. cloacae and

Escherichia coli in infected larvae. Contamination by bacteria in the 20 ml/ha of actual Lecontvirus used in spray programs would not likely exceed measurable background levels. Toxicological studies of Lecontvirus have included these impurities (ground larvae) within the evaluation, i.e. studies have evaluated the whole spectrum of components, not the purified polyhedral bodies. It is important to realize that this disease does occur in nature (though rare), being first reported as early as 1912 (see Cunningham and Entwistle 1981, p. 396). However, it was only identified as a NPV in 1951 (Steinhaus 1951). The disease in N. lecontei was first found occurring naturally in Ontario in 1950 (Bird 1961).

2.4 Spraying/Application Practices

The use of Lecontvirus in Canada is restricted for application under the direct supervision of federal or provincial forestry service personnel. The use of the product is classified as Forest Management (use on greater than 500 ha) or Woodlands Management (use on less than or equal to 500 ha). The product can be obtained only through request to the Forest Pest Management Institute. This procedure regulates users.

The material can be applied both aerially or by ground application (See label, Appendix 1). The volumes of spray mix and dosage are dependent on type of application and life stage of N. lecontei larvae (Appendix 1).

The maximum rate of spray mix applied by ground is 20 litres/ha with an effective dosage of 10×10^9 polyhedral inclusion bodies, i.e. 10 billion inclusion bodies, per hectare. This is formulated from 20 ml of Lecontvirus for each hectare treated. Water used for tank mixes should be

in a pH range of 6.0 to 7.2. If acidic, addition of NaOH is recommended (Anonymous 1987). Ground application would generally be made during mid-July to plantations of either red pine, jack pine or Scots pine, primarily less than five meters in height. Applicators would typically walk along tree rows spraying with a gas-powered mist-blower (Podgwaite et al. 1986). Applications would normally be conducted when wind conditions were less than 10 km per hour. Applicators should spray in a downwind pattern as they move through the trees. Safety helmet, goggles, gloves and a respirator are recommended, along with standard handling procedures for insecticide use, i.e. clean hands, remove contaminated clothing etc., but in 20-30°C weather, reality and comfort dictate safety precautions. Off site biologically significant drift is possible but limited to less than 100 m when doing ground application (Payne et al. 1988). The majority of red pine and jack pine plantations in Ontario will be on upland sandy soils, well removed from flowing bodies of water, lakes and ponds.

At least 353 plantations have been treated by ground application by MNR staff (Cunningham et al. 1986). The small size of most plantations and the spotty distribution of sawflies lends itself to ground applications more than to aerial applications. A summary of spray programs with Lecontvirus in Ontario over the past 5 years has been provided in Appendix 3 (Joe Churcher, Pest Control Section, Ministry of Natural Resources).

Aerial application of Lecontvirus in Ontario has been reviewed (Cunningham et al. 1986). Again, the maximum registered rate is 10 billion PIB per hectare in 9.4 litres of water. Application can be made by boom and nozzle or by Micronair atomizers. Between 1976 and 1980, 14

plantations with a total area of 175.5 ha were treated. Since 1980, 2,399 ha have been treated. This is probably a major over-estimate of usage since many plantations are "spot-treated", e.g. 4 trees in a 5 ha plantation (Churcher, per. comm.). Table I of Cunningham et al.'s 1986 paper summarizes the conditions of aerial trials. Applications would typically occur in early morning in mid-July at temperatures greater than 15°C and wind speeds less than 10 km per hour.

Off site movement of particles is possible. Again, plantation sites are typically on sandy soils generally not close to bodies of water. If we consider a worst case scenario of direct application to water, we would assume to have 10^9 PIB per ha/10,000 m or 100,000 PIB's per surface meter^2 of water. If we assume a 1 m water depth, this would represent approximately 1 PIB in every 10 ml of water as a worst case direct application to water.

The use of Lecontvirus, whether aerially or by ground, is highly effective (Cunningham et al. 1986, Podgwaite et al. 1986) in controlling N. lecontei (greater than 96% reduction in larval numbers). The control of this insect is critical for proper management of young red pine, Scots pine and jack pine plantations (Benjamin 1955). Mortality of larvae usually occurs by 15 days post-spray. Larvae generally cease feeding well in advance of mortality. Foliage protection varies with application time but is greater than 95% as measured by centimeters of defoliation per colony of sawflies. In all studies, no retreatment of plantations was required the year after treatment. The lack of retreatment may be due to live virus from the previous season or to the poor recolonization capabilities of N. lecontei.

2.5 Environmental Fate

Approximately 10 billion PIB's of Lecontvirus are sprayed per hectare. Little research on environmental fate exists since there is no residue assay method. Biological assays suggest that virus particles may exist in plantations for several years after application. With other types of NPV (i.e. the virus used for control of European spruce sawfly), the half life for the physical presence of polyhedral bodies from either pure suspensions or suspensions including larval material were 38.3 and 55.1 days respectively. Assuming a deposit of 100 PIB's, these would decline to 1.5 and 6.2 PIB's by 240 days when the next larval generation would begin. Decay of PIB's, as measured by biological assays, appears more rapid and is likely due to the viricidal effects of ultraviolet light (see Cunningham and Entwistle 1981, p. 383). At present, all registered baculoviruses in the U.S. and Canada have been granted exemptions from the requirement of a tolerance for residues (see Shieh and Bohmfalk 1980, p. 359).

Since the virus exists naturally, it must be capable of overwintering. Corpses of infected larvae adhering to trees and NPV-contaminated bird faeces are a possible source. Baculoviruses can persist for several years in soil and it is reasonable to assume that Lecontvirus can also (see Cunningham and Entwistle 1981, p. 384). The stability of Lecontvirus in water is unknown.

2.6 Host Specificity

An excellent review of safety tests associated with these viruses has

been published (Burges et al. 1980). Key points of note from the review are:

1. Baculoviruses are unable to replicate in microorganisms, non-insect invertebrate cell lines, vertebrate cell lines, plants and non-arthropod invertebrates and vertebrates.

2. Replication in insects, outside of the particular insect family in which the virus was originally found, is rare.

3. Naturally occurring infections outside this host range have not been found.

4. Medical, veterinary and phytopathology literature contains no reference to Baculoviridae affecting man, animals or plants.

5. At times of natural epizootics of caterpillars, birds have been found to have 18% by weight inclusion bodies in faeces without detrimental effect (see Burges et al. 1980, p. 330).

6. Exposure of 10^6 to 10^9 PIB/animal, i.e. shrimp, oysters, water fleas (Daphnia sp.), failed to demonstrate an effect.

7. In vertebrates, challenge studies representing 10 to 100 times field exposure, i.e. 1×10^9 intradermal or 20×10^9 PIB/kg, showed no effect.

8. NPVs have no effect on mammalian chromosomes.

In reviewing Lecontvirus, the large body of information on Baculoviridae adds supportive evidence to safety tests specific to Lecontvirus. Specific studies related to Lecontvirus are summarized in Table 1. In addition, comparison of restriction enzyme profiles for the original Lecontvirus isolates (ca. 1976) and the virus currently being produced shows no change in the viral genome. This demonstrates the genetic stability of Lecontvirus (Dr. B.M. Arif, per. comm. 1988).

Table 1. Safety tests with Lecontvirus involving animals.

Species	Type of Test	Dosage/Exposure Rate	Results
Chickens/ turkeys	Intubation	1.4×10^6 PIB's per g body weight	No effect (Valli et al. 1976)
Rats/ rabbits	Intubation	3×10^6 PIB's per g body weight	No effect (Forsberg et al. 1978)
Rats	Multiple dose	3.75×10^8 PIB's over 90 days	No effect (Forsberg et al. 1978)
Rabbits	Dermal exposure	4.2×10^9 PIB's per animal	No effect (Forsberg et al. 1978)
Hamsters	Inhalation	2.72×10^8 PIB's per animal/ 1 hour	No effect (Forsberg et al. 1978)
Rabbits	Eye irritation	4.2×10^9 PIB's per eye	No effect (Forsberg et al. 1978)
Birds e.g. sparrow grosbeak flicker oriole	Aerial application	5.5×10^9 PIB/ha	No effect (Kingsbury et al. 1978)
Honeybees	Aerial application	5.5×10^9 PIB/ha	No effect (Kingsbury et al. 1978)
Aquatic inverte-brates	Aerial application	5.5×10^9 PIB/ha	No effect (Kingsbury et al. 1978)
Rainbow trout	Ingestion	3×10^6 PIB's per g body weight	No effect (Hicks et al. 1981)
Rainbow trout	Water-borne virus	24,000 PIB/ml water	No effect (Hicks et al. 1981)
Daphnia pulex	Water-borne virus	24,000 PIB/ml water	No effect (Hicks et al. 1981)
Guinea pig	Hyper-sensitivity	10 injections, 1 every 48 hours at 2×10^7 PIB's per injection	Initial flare reaction, no evidence of hyper-sensitivity (Cunningham 1988, personal communication)

The only confirmed effect noted from baculoviruses on vertebrates has been slight skin irritation or eye irritation in rabbits with viruses other than Lecontvirus. The effect is believed to be due to the insect material associated with the virus, and not to the virus itself.

2.7 Virulence

Lecontvirus is only effective against the red-headed pine sawfly, N. lecontei. Infections of N. sertifer and N. abietis with Lecontvirus will occur but at high concentrations of virus application with little resultant mortality (see Cunningham and Entwistle 1981, p. 381). No organisms outside of the family Diprionidae are known to be affected.

All baculoviruses must be ingested by larvae to initiate infection. The alkaline pH of the larval gut dissolves the protein inclusion bodies to release infectious virions. These virions penetrate the cells of the gut and initiate cellular infection. Nucleopolyhedrosis viruses will replicate only in midgut epithelial cells. Polyhedral inclusion bodies are produced and within 10 to 12 days, the larva dies. As the larvae disintegrate, polyhedral bodies are released into the forest. Live virus in bird faeces have been identified and birds feeding on infected larvae may aid in the dissemination of this virus (see Cunningham and Entwistle 1981, p. 383). The infective dose required to kill 50% of the population is extremely difficult to determine, but is believed to range from 20 PIB's per first instar larva to 800 PIB's for early fifth instars (see Cunningham and Entwistle 1981, p. 389). The toxicity of Lecontvirus is measured as the number of PIB's per ml of product, e.g. 5×10^8 PIB's/ml.

2.8 Aquatic Toxicity

Due to the specificity of Lecontvirus, few studies on the toxicity to aquatic organisms exist. All available data related to Lecontvirus and baculoviruses in general indicate no toxicity to aquatic organisms.

In general, Baculoviridae have been shown not to replicate in grass shrimp, brown shrimp, oysters and water fleas (at dosages of 10^6 to 10^9 PIB/animal, see Burges et al. 1980). Seven species of fish have been exposed to Heliothis NPV without effect (see Burges et al. 1980). Tests with nuclear polyhedrosis virus affecting Neodiprion sertifer did not affect blue gills or rainbow trout (Cunningham and Entwistle 1981, p.393).

The most extensive aquatic toxicity studies done on Lecontvirus are by Hicks et al. (1981). The key points are as follows:

A. Rainbow Trout Ingestion Study:

The materials tested against rainbow trout were: 1) lyophilized, Lecontvirus-infected N. lecontei (sawflies); 2) lyophilized, unaffected N. lecontei; 3) purified polyhedral inclusion bodies of Lecontvirus; and 4) control.

A challenge test of 3 million PIB's/g body weight of rainbow trout was used for evaluation. Fish weighed an average of 109 g.

Results:

1. no lesions observed in any of the 13 tissues examined.
2. some incidental abnormalities in all groups.
3. no significant differences in weight at any time during study.
4. no abnormalities in behaviour of fish observed.

B. Rainbow Trout Water-borne Virus Study:

Thirty-six rainbow trout were subjected to the following treatments: 1) lyophilized, Lecontvirus-infected sawfly larvae; 2) purified PIB's of Lecontvirus; and 3) control.

The water was treated at 24,000 PIB per ml (i.e. 240,000 x hypothetical field dose).

The aquaria were left as closed systems for 7 days, with the remaining 21 days at a continual flow of 2.3 l/min.

Results:

1. no lesions observed attributable to treatment.
2. no significant difference in initial, weekly or final weights of fish.
3. no abnormalities in behaviour.

C. Daphnia pulex Study:

A cohort of 60 Daphnia pulex were fed algae, and each treatment group of Daphnia put in 100 ml H_2O. Treatments were as follows: 1) lyophilized Lecontvirus-infected N. lecontei; 2) lyophilized, unaffected N. lecontei; 3) control. Twenty Daphnia received each treatment.

Daphnia were exposed to 24,000 PIB/ml water, and samples (consisting of 4 Daphnia) were taken at 0, 1, 3, 7, and 14 days post-inoculation.

The progeny of Daphnia were counted, and Daphnia also underwent microscopic examination.

Results:

1. no significant difference in brood size or fecundity of Daphnia.
2. no lesions or abnormalities in any of the tissues examined.

The overall conclusions were that "under conditions of this study, rainbow trout and Daphnia were unaffected ... at dosages far in excess of amounts of material which could get into ponds or streams." Similarly Kingsbury et al. (1978) failed to detect impact to aquatic invertebrates in operational field trials.

2.9 Health Effects (to Humans)

Specific studies related to human susceptibility of Lecontvirus do not exist. Extrapolations from animal toxicology tests are used to predict potential human health effects. With Lecontvirus, there was no effect in the following studies (refer to Table 1 for dosages):

1. acute oral tests on rabbits and rats (dosage was equivalent to the amount of product required to spray 40 ha administered to a 70 kg man, i.e. 3×10^6 PIB's/g body weight).
2. acute dermal tests on rabbits.
3. multiple-dose, 90-day exposure of rats.
4. acute eye irritation of rabbits.
5. inhalation tests in hamsters.

In mice, mortality by ingestion was due to the physical effects of forced feeding (see Cunningham and Entwistle 1981, p. 393). They were killed at a rate equivalent to the amount of product required to spray 2400 ha administered to a 70 kg man.

Actual human data does exist for other baculoviruses. With Heliothis NPV, studies have been done with humans at dosages from 6×10^9 to 3×10^{12} PIB/kg. Skin irritation studies with dosages up to 10^7 PIB/mm^2 of

skin have been done. Ten humans were fed 10^9 PIB/day for 5 days (see Burges et al. 1980, pp. 331-332). All of these studies showed no effect.

If we consider 100 PIB's of Lecontvirus would be in a litre of water, the feeding dosages of Heliothis virus would be equivalent to drinking 10^7 litres of water. Skin sensitivity tests represent even higher levels.

From available animal data specific to Lecontvirus, human data available related to other baculoviruses, and the margins of safety tested, there appears to be no concern for humans from Lecontvirus.

2.10 Effects on Non-target Terrestrial Animals

Mammals: The possibility of effect of Lecontvirus to terrestrial animals is predicted by the laboratory toxicology studies already mentioned (see Forsberg et al. 1978 for mammalian studies on rats, mice, rabbits and hamsters). The summary of this study is that: "From these studies it can be concluded that the NPV of Neodiprion lecontei was without effect on laboratory animals housed in a controlled environment and including a group of animals which were immunosuppressed. Since no indication of NPV infection or increased susceptibility to other infections or significant change from accepted values was caused by these preparations by any route we conclude that this NPV is not a hazard to mammals."

Birds: Valli and Claxton (1976) evaluated oral toxicity to chickens and turkeys in a laboratory study. The summary of this study is: "In summary, I feel the intensity with which these birds were studied and lack of variation occurring between the test and control groups indicates that the nuclear polyhedrosis virus of the redheaded pine sawfly does not establish

infection in these birds or cause any injury. It therefore appears to constitute no hazard whatever to these avian species."

During natural invertebrate virus epizootics, the exposure of some vertebrates has been massive, e.g. 18% by weight of inclusion bodies were found in faeces of birds that preyed on infected caterpillars (see Burges et al. 1980, p. 330). There was no indication of bird mortality.

Field Studies: A field evaluation of Lecontvirus at 5.5×10^9 PIB/ha has been done by Kingsbury et al. (1978) in Renfrew, Ontario. This study evaluated the impact on birds (many species present), honey bees and aquatic fauna. The conclusions of the study are that: "From the data collected and the field observations, it would appear there were no immediate undesirable effects due to the NPV spray application on the avian or aquatic fauna nor on colonies of honey bees...The honey bees suffered no discernable losses, although further studies would be necessary to determine if there had been any rejection of contaminated pollen by the bees. There were some anomalies in the control stream which made the aquatic study less deterministic than ideal, but except for unexplained decreases in chironomid and mayfly counts the benthic fauna was clearly unaffected." Natural variations found in populations within and between samples would readily explain the benthic observations related to chironomids and mayflies.

In addition, results from other tests with baculoviruses indicate no effect to birds, mammals, fish, and honeybees (Burges et al. 1980). The potential effects to amphibians and reptiles are unknown, but there have been no reported impacts from field studies using baculoviruses.

2.11 Methods for Enumerating Lecontvirus in Aquatic Samples

At present, there is no analytical method for enumerating Lecontvirus in water. Biochemical studies on purified PIB's for Lecontvirus show that the PIB protein has a molecular weight of 28,000 Daltons. The virions have 9 structural proteins ranging from 7,600 to 96,700 Daltons (see Cunningham and Entwistle 1981, p. 392).

In the past, microscopic analysis of fresh, dissected gut material has been used to detect NPVs. Viruses can be diagnosed only at 1500x or greater magnification.

Enzyme-linked immunosorbent assay (ELISA) will allow for identification of NPV virus in infected larvae. This technique combines the use of immobilised antigens or antibodies on a solid phase with enzyme-labelled antibody or antigen conjugates to yield assays with the sensitivity and specificity of isotopic techniques (Voller and de Savigny 1981). Ten nanograms of polyhedral protein can be detected, and larvae containing 5×10^5 PIB's or more give positive reactions (see Cunningham and Entwistle 1981, p. 392).

3.0 LITERATURE CITED

Anonymous. 1987. Nuclear Polyhedrosis viruses- Technical reference. Forest Pest Management Institute, Box 490, Sault Ste. Marie, Ontario. 4pp.

Benjamin, D.M. 1955. The biology and ecology of the red-headed pine sawfly. USDA For. Serv. Tech. Bull. 1118. 57 pp.

Bird, F.T. 1961. Transmission of some insect viruses with particular reference to ovarial transmission and its importance in the development of epizootics. J. Insect. Path. 3: 352-380.

Burges, H.D., G. Croizier and J. Huber. 1980. A review of safety tests on baculoviruses. Entomophaga 25: 329-39.

Cunningham, J.C., P. de Groot and W.J. Kaupp. 1986. A review of aerial spray trials with Lecontvirus for control of redheaded pine sawfly, Neodiprion lecontei (Hymenoptera: Diprionidae), in Ontario. Proc. Ent. Soc. Ont. 117: 65-72.

Cunningham, J.C. and P.F. Entwistle. 1981. Control of sawflies by baculovirus. In: Microbial control of pests and plant diseases 1970-80. H.D. Burges, ed. Academic Press, London. pp. 379-407.

Cunningham, J.C. and J.R. McPhee. 1986. Production of sawfly viruses in plantations. Can. For. Serv., Sault Ste. Marie, Tech. Note No. 4. 4 pp.

Forsberg, C.M., V.E.O. Valli and P. Dwyer. 1978. Mammalian pathogenicity of the nuclear polyhedrosis virus of Neodiprion lecontei. Can. For. Serv., Sault Ste. Marie, Contract Rep. OSU76-00226. 524 pp. + appendices.

Hicks, B.D., J.R. Geraci, J.C. Cunningham and B.M. Arif. 1981. Effects of red-headed pine sawfly, Neodiprion lecontei, nuclear polyhedrosis virus on rainbow trout, Salmo gairdneri, and Daphnia pulex. J. Environ. Sci. Hlth. B16: 493-509.

Kingsbury, P., B. McLeod and K. Mortensen. 1978. Impact of applications of the nuclear polyhedrosis virus of the red-headed pine sawfly, Neodiprion lecontei (Fitch), on non-target organisms in 1977. Can. For. Serv., Sault Ste. Marie, Inf. Rep. FPM-X-11. 27 pp.

Payne, N., B.V. Helson, K.M.S. Sundaram and R.A. Fleming. 1988. Estimating buffers for spray applications. Pest. Sci. (in press).

Podgwaite, J.D., P. Rush, D. Hall and G.S. Walton. 1986. Field evaluation of a nucleopolyhedrosis virus for control of redheaded pine sawfly (Hymenoptera: Diprionidae). J. Econ. Ent. 79: 1648-1652.

Shieh, T.R. and G.T. Bohmfalk. 1980. Production and efficacy of baculoviruses. In: Symposium on microbial agents for the control of insect pests. Biotech. Bioeng. 22: 1357-1375.

Steinhaus, E.A. 1951. Report on diagnosis of diseased insects 1944-1950. Hilgardia 20: 629-678.

Valli, V.E.O. and M.J. Claxton. 1976. Avian toxicity test of the nuclear polyhedrosis virus of the redheaded pine sawfly Neodiprion lecontei. Can. For. Serv., Sault Ste. Marie, Contract. Rep. OSS5-0194. 237 pp.

Voller, A. and D. de Savigny. 1981. Enzyme linked immunosorbent assay (ELISA). In: Techniques in clinical immunology. R.A. Thompson ed. Blackwell Scientific Publications, 2nd ed. Oxford. pp. 157-169.

LECONTVIRUS

Nuclear Polyhedrosis Virus

BIOLOGICAL INSECTICIDE FOR REDHEADED PINE SAWFLY (NEODIPRION LECONTEI)

FOR FOREST WOODLAND AND ORNAMENTAL USE

EMULSIFIABLE OIL CONCENTRATE

RESTRICTED

GUARANTEE Polyhedral inclusion bodies of redheaded pine sawfly nuclear polyhedrosis virus 0.0015%
(Contains at least 5×10^8 polyhedral inclusion bodies per millilitre)

READ LABEL BEFORE USING

CAUTION SKIN and EYE IRRITANT. Avoid contact with skin, eyes or clothing. Avoid inhalation.

REGISTRATION NUMBER 17824 PEST CONTROL PRODUCTS ACT

NET CONTENTS _____ ML

USE BEFORE _____

FOREST PEST MANAGEMENT INSTITUTE
Canadian Forestry Service
P.O. Box 490
Sault Ste. Marie, Ontario
P6A 5M7

NOTICE TO USER: RESTRICTED. This control product is to be used in accordance with the directions on this label. It is an offence under the Pest Control Products Act to use a control product under unsafe conditions.

NATURE OF RESTRICTION: This product is to be used only in the manner authorized; consult local pesticide regulatory authorities about use permits which may be required.

RESTRICTED USE

This product is to be used only under the direct supervision of Federal or Provincial Forestry Service personnel.

DIRECTIONS FOR USE

Lecontvirus is a highly selective insecticide for the control of redheaded pine sawfly larvae. Applications should be made when most of the eggs have hatched and larvae are in the first and second instars. It is also effective on third instar larvae, but should not be applied after the majority of larvae reach the fourth instar. A higher dosage is recommended for third and fourth instar larvae. Fourth instar larvae can easily be recognized by black spots which are not present on the earlier instars. Larvae must eat polyhedra to become infected. If one larva in a colony becomes infected, it will transmit the disease to the rest of the larvae in that colony.

The emulsifiable oil concentrate contains virus-infected redheaded pine sawfly larvae which have been freeze-dried and ground to a fine powder. Material tends to settle on the bottom of the container.

SHAKE BOTTLE WELL AND ENSURE ALL SEDIMENT IS RESUSPENDED BEFORE MEASURING OUT REQUIRED DOSAGE.

AERIAL APPLICATION

For aerial application, it is recommended that Lecontvirus be used with conventional boom and nozzle or Micronair® spinning nozzles on fixed wing aircraft or Beecomist® spinning nozzles with drilled sleeves on helicopters. Aircraft should be calibrated to deliver droplet diameters of 100-250 microns. To ensure a good deposit, Lecontvirus should be applied only under conditions of highest possible relative humidity and low wind velocity, i.e., in the early morning or early evening when good spray conditions prevail. Do not mix Lecontvirus with any materials other than water.

RATES FOR AERIAL APPLICATION

	Larval instar	RATE OF LECONTVIRUS per hectare		WATER*	TOTAL EMITTED volume per hectare
		Millilitres	PIB	Litres	Litres
Plantations	1st & 2nd	10	5×10^9 (5 billion polyhedral inclusion bodies)	9.4	9.4
	3rd & 4th	20	10×10^9 (10 billion polyhedral inclusion bodies)	9.4	9.4

GROUND APPLICATION

This product may be applied from the ground with a variety of equipment and using a variety of techniques. With backpack mistblowers, every third or fourth row in a plantation can be treated taking advantage of any wind to carry the spray cloud across rows. With pressurized handheld hydraulic sprayers, individual colonies can be sprayed. Here particular care should be taken to treat those at the tops of the trees as virus will spread to those located lower on the trees. Usually redheaded pine sawfly are not evenly distributed throughout plantations and particular attention should be given to obtaining good spray coverage on "hot spots". Do not mix Lecontvirus with any materials other than water.

RATES FOR GROUND APPLICATION

	Larval instar	RATE OF LECONTVIRUS per hectare		WATER*	TOTAL EMITTED volume per hectare
		Millilitres	PIB	Litres	Litres
Plantations	1st & 2nd	10	5×10^9 (5 billion polyhedral inclusion bodies)	20	20
	3rd & 4th	20	10×10^9 (10 billion polyhedral inclusion bodies)	20	20

MIXING FOR AERIAL AND GROUND APPLICATIONS

Thoroughly shake the bottle of emulsifiable oil concentrate and add the required volume to the appropriate volume of water*. Agitate thoroughly. With mistblowers and handheld pressure sprayers mix material directly in their tanks. Mix only the amount of material to be sprayed that day and do not store the final diluted preparation.

PRECAUTIONS

Keep Out of Reach of Children.

Skin and Eye Irritant: Avoid contact with skin, eyes or clothing. In case of contact, immediately flush eyes or skin with plenty of water. Get medical attention if irritation persists. Avoid inhalation. Wear mask or respirator during preparation and application of this material. Do not spray into lakes, streams or ponds. Do not contaminate any body of water by cleaning of equipment or disposal of wastes. Do not reuse empty container. Destroy or discard in safe place. Do not store at temperatures above 32°C.

Limitation of Warranty: Guarantee shall be limited to the terms set out on the label and subject thereto, the user assumes the risk to persons or property arising from the use or handling of this product and accepts the product on that condition.

* Chlorinated or highly alkaline water should not be used in tank mixes. Tank mix pH should be between 5.0 and 8.0. If only chlorinated water is available, it should be allowed to stand for 24 hours before use.

Government of Canada / Gouvernement du Canada
Canadian Forestry Service / Service canadien des forêts

Appendix 2

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TECHNICAL NOTE

Forest Pest Management Institute

No. 4 September 1986 Laboratory Methods

PRODUCTION OF SAWFLY VIRUSES IN PLANTATIONS

Introduction

Two viral insecticides for control of sawflies are produced at the Forest Pest Management Institute for distribution to clients. These are Lecontvirus for control of redheaded pine sawfly, *Neodiprion lecontei*, which was registered under the Pest Control Products Act (Canada) in 1983 and Sertifervirus for European pine sawfly, *N. sertifer*. A registration petition for Sertifervirus is currently being evaluated.

There are no artificial diets available for sawflies. In the laboratory, these insects must be reared on fresh foliage. This is time consuming and virtually precludes handling large numbers of larvae. Nuclear polyhedrosis viruses (NPVs) that infect sawflies have been propagated since the early 1950's in heavily-infested plantations. Sawfly larvae are gregarious and feed in colonies, greatly simplifying harvesting of diseased and dead larvae. Methods have been refined over the years and current techniques are outlined below. Procedures are identical for production of both Lecontvirus and Sertifervirus.

Selecting sites

The aim of a virus production program is to harvest diseased and dead larvae when they are fully grown and yield the greatest amount of virus. Under these circumstances, defoliation and damage are anticipated on infested trees, which may be unacceptable. Ideal sites are plantations that have a history of severe damage and are virtually "write-offs", abandoned Christmas tree farms or plantations with trees large enough to withstand moderate defoliation. Areas naturally infested with sawflies are the first choice as propagation sites, but if inconveniently located, colonies of sawflies can be transferred to a more suitable location by clipping twigs with colonies of larvae, transporting them in paper bags and then tying the twigs with larvae on to other trees using "twist ties" (Fig. 1). As the cut foliage dries out, larvae migrate on to the new host tree.

Timing of virus infection and dosage

Larvae in the fourth-instar are sprayed with virus. An aqueous suspension containing 10^6 polyhedral inclusion bodies (PIB)/mL is applied. This is a higher dosage than is used for control operations when, ideally, first- and second-instar larvae are treated. When infested plantations are used as propagation sites, a mistblower is used, and every third or fourth row is sprayed (Fig. 2). Volume applied is about 20 L/ha.

When individual colonies are transferred to new host trees, they are marked with flagging tape for fast and easy detection. Here, the individual colonies are sprayed with an atomizer spray bottle (Fig. 3). Both the actual colony and surrounding foliage are sprayed: each colony receives about 5 mL of suspension.

Harvesting

The length of time between spraying and death of larvae depends on the ambient temperature and the first check is made at 8 days post-spray. When a plantation is sprayed with a mistblower, mortality occurs over a prolonged period, usually between 8 and 20 days post-spray. When a hand-sprayer is used to treat individual colonies mortality is more uniform, occurring between 8 and 12 days post-spray. Twigs with colonies containing dead and diseased larvae are clipped from the trees and placed in paper bags. Healthy larvae rear up when disturbed and colonies with active larvae are left so that the infection process will proceed. Colonies of larvae are harvested daily, otherwise dead larvae will be removed by predacious and scavenging insects or washed off the foliage by rain.

Bags containing diseased and dead colonies on foliage are stapled shut and kept cool until larvae can be picked off the foliage with forceps and placed in plastic petri dishes (Fig. 4). Larvae that are alive and active are placed in plastic boxes on fresh foliage and reared at room temperature until they either die or pupate. Petri dishes containing dead larvae are frozen and stored at -20°C until processed.

Processing

Frozen, NPV-infected and NPV-killed larvae are freeze-dried and ground to powder for 30 seconds in a Waring blender. To obtain a finer powder, which will pass through a 20 mesh sieve when suspended in water or oil, the ground larvae are mixed with an equal amount of crushed dry-ice and re-ground for 30 seconds in a blender. The powdered material is then stored in tightly sealed containers at 4°C until required for bio-control operations.

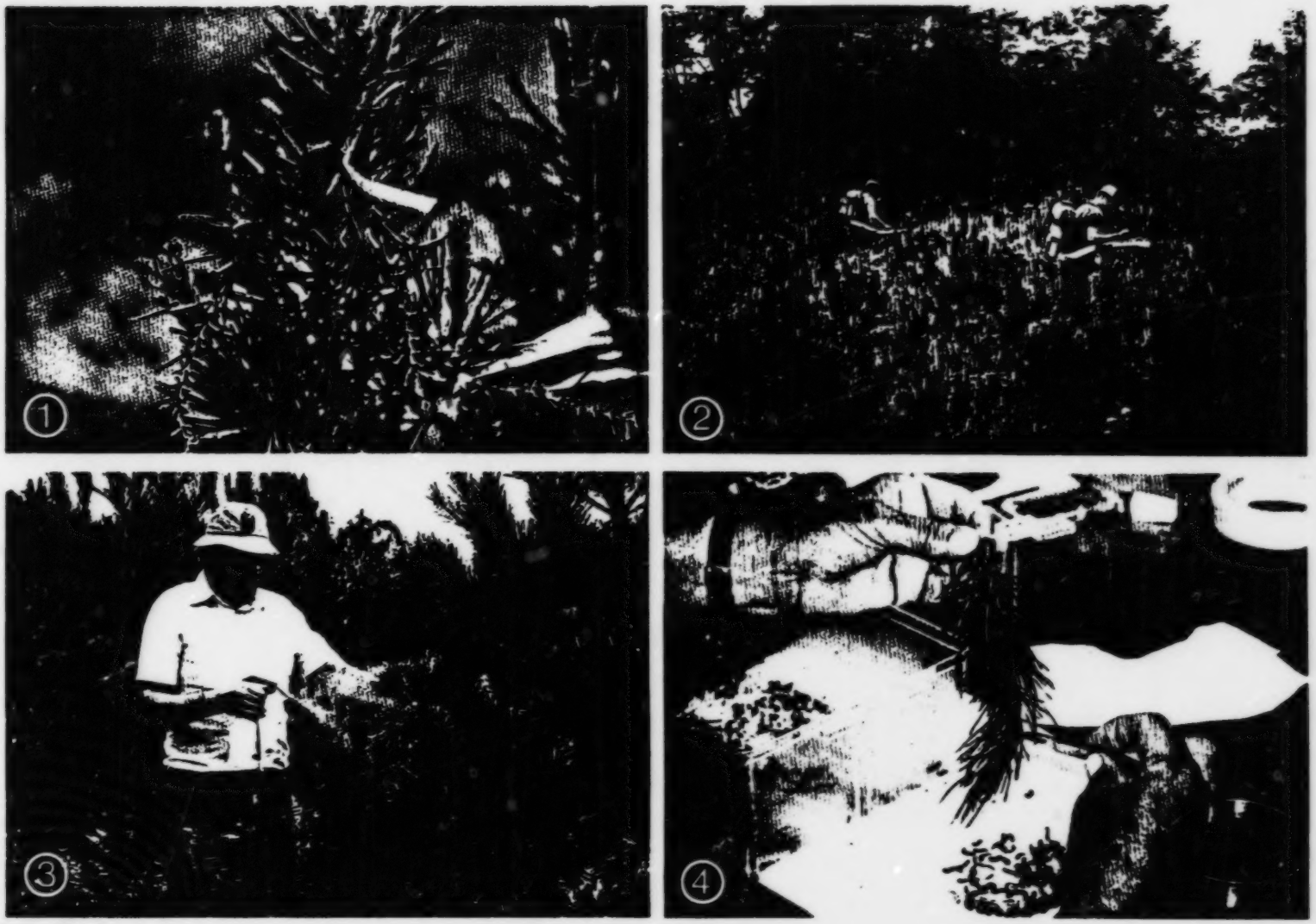
The potency of each batch is determined by estimating the number of polyhedral inclusion bodies per gram using the dry counting technique described by Wigley (1980). If larvae are virus-killed or heavily diseased when harvested, the powder contains about 2 x 10^{10} PIB/g. The dead larvae contain some bacteria which are of no concern. However, a quality control check is run to determine if the bacteria are at an acceptable level and that no human pathogens are present. Quality control procedures are described by Podgwaite and Bruen (1978).

Currently, material is shipped to clients as an emulsifiable oil concentrate. The oil used is Abbott Laboratories gelled oil vehicle which is the carrier for *Bacillus thuringiensis* in their product called Dipel 88®. The shelf-life of the NPV in this oil is less than one year and a search is being made for an alternative vehicle. We only formulate sufficient virus to use for one season and recommend that surplus material be discarded.

Conclusions

Production costs of Sertifervirus and Lecontvirus are very low; the main factors are salaries, travelling time to production sites, lodging and related expenses. It takes about 50 virus-infected larvae to produce sufficient material to treat 1 ha. If produced close to the laboratory with no overnight accommodation involved, costs range as low as $.50/ha. Production at a distant site could raise this price to $2.50/ha.

Far greater quantities of material can be produced in naturally infested plantations than by moving colonies of larvae to other sites. In 1984, 6 red and jack pine plantations with a combined area of 9.5 ha yielded 1.0 kg of Lecontvirus which is sufficient to treat 2,000



Figs. 1-4. 1) Colony of European pine sawfly is tied onto a Scots pine with a "twist tie". 2) A red pine plantation infested with redheaded pine sawfly is sprayed with virus using mistblowers. 3) Colonies of redheaded pine sawfly transferred to a convenient location are individually sprayed with an atomizer spray bottle. 4) NPV-killed European pine sawfly larvae are removed from foliage and placed in a petri dish.

ha. In 1985, 200 colonies of European pine sawfly, transferred to suitably located host trees, yielded 50 g of Sertifervirus which is sufficient to treat 100 ha.

We have only produced NPVs for control of *N. sertifer* and *N. lecontei*, but these methods could be adopted for other colonial species of Diprionid sawflies known to be susceptible to NPVs. These include Swaine's jack pine sawfly, *N. swainei*, red pine sawfly, *N. nanulus nanulus*, balsam fir sawfly, *N. abietis* and the jack pine sawflies *N. pratti banksianae* and *N. pratti paradoxicus*.

References

Podgwaite, J.; Bruen, R.B. 1978. Procedures for the microbiological examination of production batches of the nuclear polyhedrosis virus (Baculovirus) of the gypsy moth, *Lymantria dispar* L. USDA For. Serv. Gen. Tech. Rep. NE 38. 8 p.

Wigley, P.J. 1980. Practical: counting micro-organisms. Pages 29-35 *in* Microbial Control of Insect Pests (J. Kalmakoff and J.F. Longworth Comp.). N.Z. Dep. Sci. Ind. Res. Bull. 228.

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Appendix 3. Applications of Lecontvirus (Red-headed pine sawfly NPV) in Ontario by MNR, 1980-1986.

Year	Total Ha Treated	Ha Treated With NPV	Application Rate
1980	1,037	585	5 - 10 X 10^9 PIB/20L/ha
1981	939	492	"
1982	914	691	"
1983	200	200	"
1984	23	22	"
1985	103	103	"
1986	477	306	"